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Overview

Sheep Model in Orthopedic Research: A Literature Review

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The aim of the study reported here was to provide some basic and general information on the suitability of an experimental sheep model for conducting in vivo orthopedic studies. The authors have classified the fundamental aspects that should be carefully evaluated when using sheep as an experimental model in orthopedic research: factors strictly related to bone anatomy and formation; and factors strictly affecting bone physiology, such as gastrointestinal mineral and vitamin absorption, and reproductive cycle. Future investigations should address all of the aspects highlighted, since there is no animal with the same anatomic, biochemical, physiologic, and biological characteristics as those of human beings. Moreover, useful data for treating orthopedic patients are based not only on good planning and study design, but also on perfect knowledge of the animal used and the differences between the model and the human being. The authors hope that this report will contribute to extrapolation of reliable data for use of sheep in the orthopedics field.

Background

Animal models have been widely used in biomedical research and have been crucial for acquiring basic science and clinical knowledge pertaining to medical science. In recent years, much information has been provided on surgical or medical treatment of orthopedic pathologic conditions and traumas (1-4). Even though various in vitro models have been successfully used and in vivo experiments are gradually giving way to in vitro alternative techniques, their usefulness is limited to the investigation of some complicated biomedical issues. Complete replacement of animals with suitable non-animal alternatives still remains a long-term goal. Unfortunately, animal models are not as standardized as are most of the in vitro methods, and sometimes there is not a well established relationship between the choice of the animal to be used experimentally and the kind of study to be performed.

Despite optimal planning, experimental design and equipment, selecting an inappropriate animal model can lead to seriously misleading information. In brief, the animal model for experimental use should be carefully assessed to ensure analogy with humans, avoid excess use of animals as stated by various international guidelines, and prevent waste of time, efforts, and funding. The ultimate decision on the relevance of any model should focus on the similarity between the human condition and the hypothesis being tested.

So far, various animal species have been used as models of human orthopedic pathologic conditions. The current computerized literature search of bibliographic databases (MEDLINE), according to the strategy detailed in the appendix, has identified a total of 21,500 of 237,903 studies based on animal species, complete with abstract in the field of orthopedic and traumatology published from 1970–2001. Fig. 1 reveals the results obtained, expressed as percentages and subdivided according to the following categories of animal species used: non-human primates, dogs, cats, pigs, cattle, horses, sheep, goats, rabbits, guinea pigs, rats, and mice. The most commonly used laboratory animals have turned out to be rats, mice, and rabbits, probably because they are cheaper and easy to handle. However, rats and mice are small, and many of the studies involving these animal models are about basic orthopedics (toxicologic tests on biomaterials). Moreover, their use requires special instrumentation and surgical techniques. In addition, the rate of bone repair is known to be inversely related to the position of the species on a phylogenetic scale, and rats have higher capacity of bone regeneration than do humans (5). However, use of small and cheap animals could be accepted in the early stages of the test, whereas the healing characteristics of the animals should approximate those of humans (6) during the late stages. When the aim of the study is to examine some surgical and orthopedic issues, such as fixation of fractures, osteotomies, and repair of ligaments and cartilage defects, the kind of animal selected, in particular its size and anatomy, should fully meet the needs of the experimental protocol (6, 7). These studies require animal models with limbs and skeletal segments of adequate size, as similar as possible to those of the human beings, to find surgical solutions and solve mechanical problems by applying the same instrumentation as that used in humans. Therefore, the evaluation of final orthopedic and prosthetic devices requires large-sized animals, such as non-human primates, dogs, pigs, sheep, and goats.

More detailed analysis of the percentage use of some species during each decade considered for this study (1970–2001) has revealed a modest decrease in the use of non-human primates, dogs and rabbits, as well as a very modest increase in the use of goats, sheep, and pigs (Fig. 2).

Diagrams in Fig. 3 show use of the aforementioned species for

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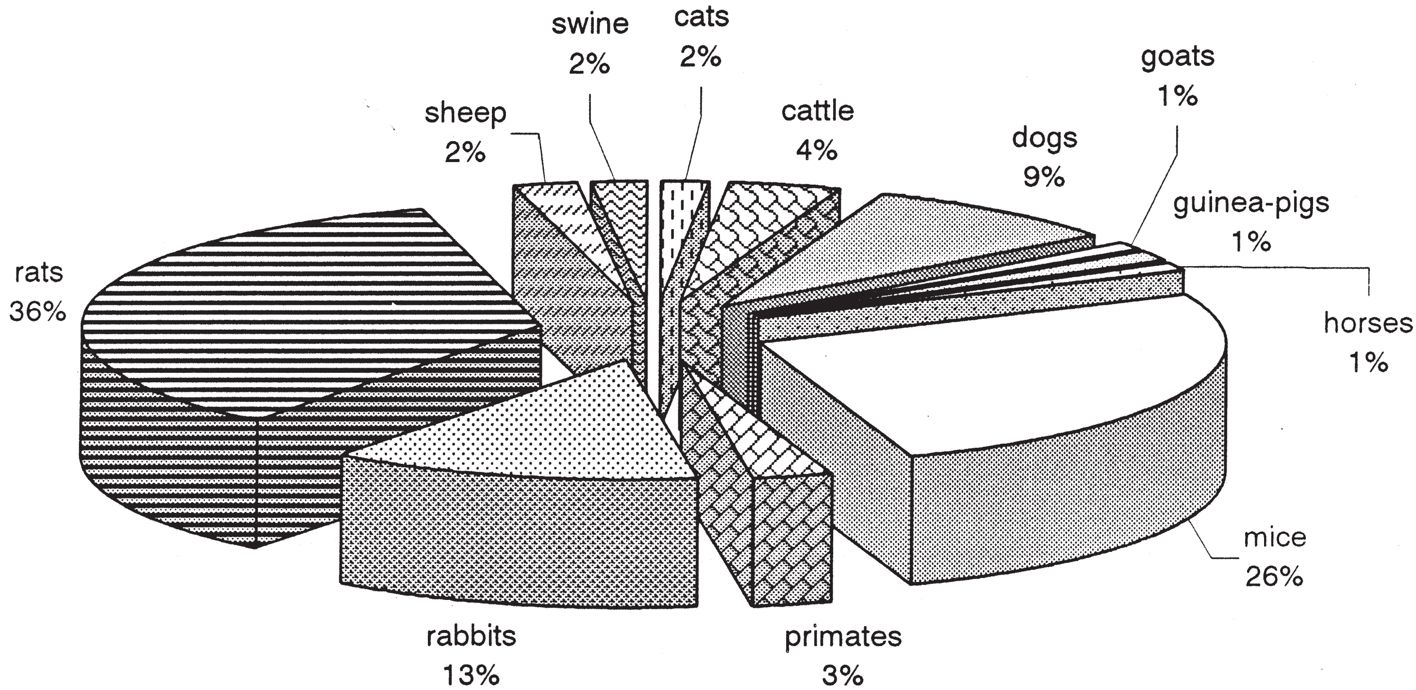

Figure 1. Mammals used for orthopedic research during 1970–2001. Average percentage expressed on the basis of total amount of 21,500 studies of animal species reported in the field of orthopedics.

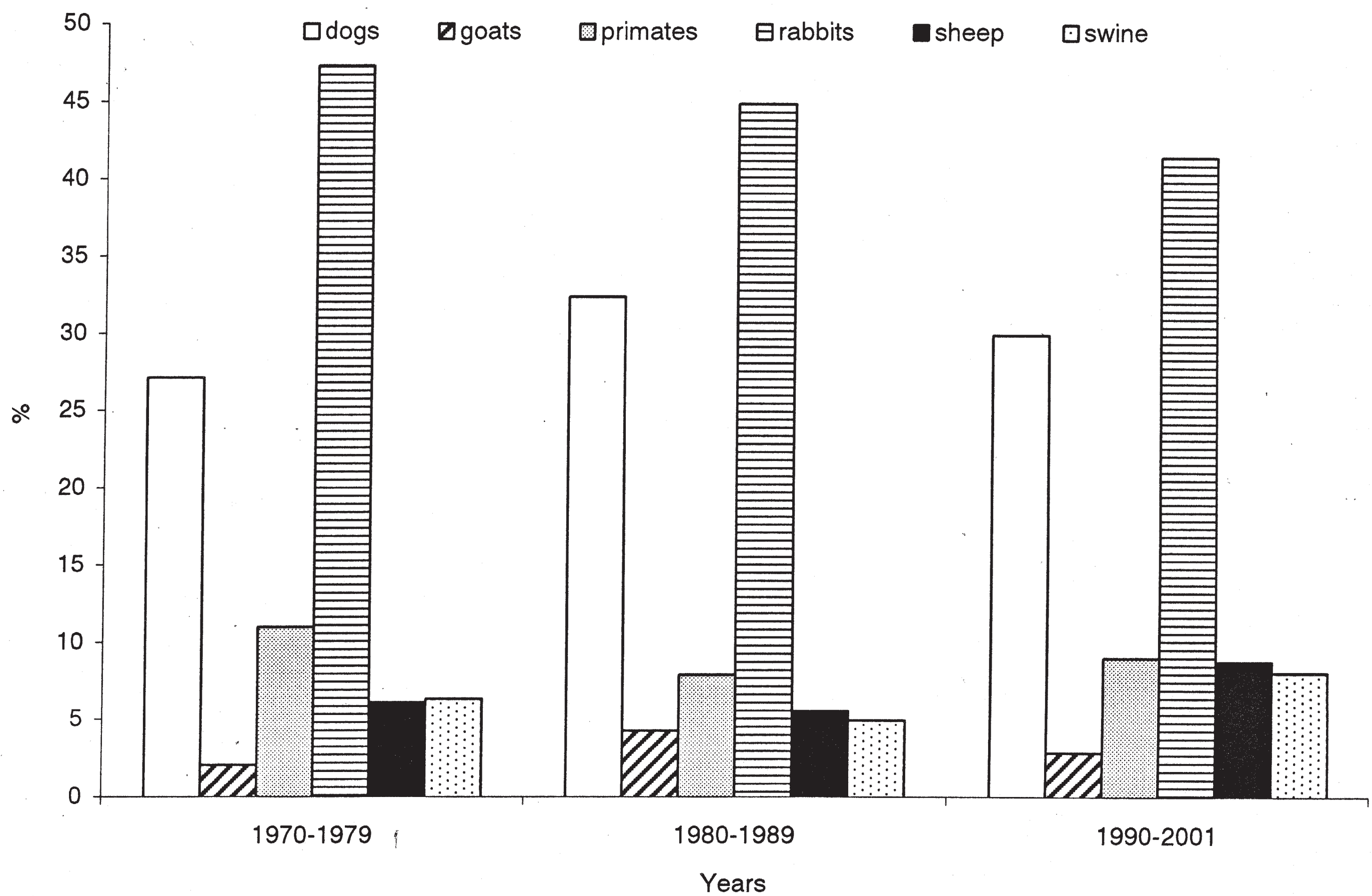

Figure 2. Average percentage use of some species during each decade considered (1970–2001) in the field of orthopedics.

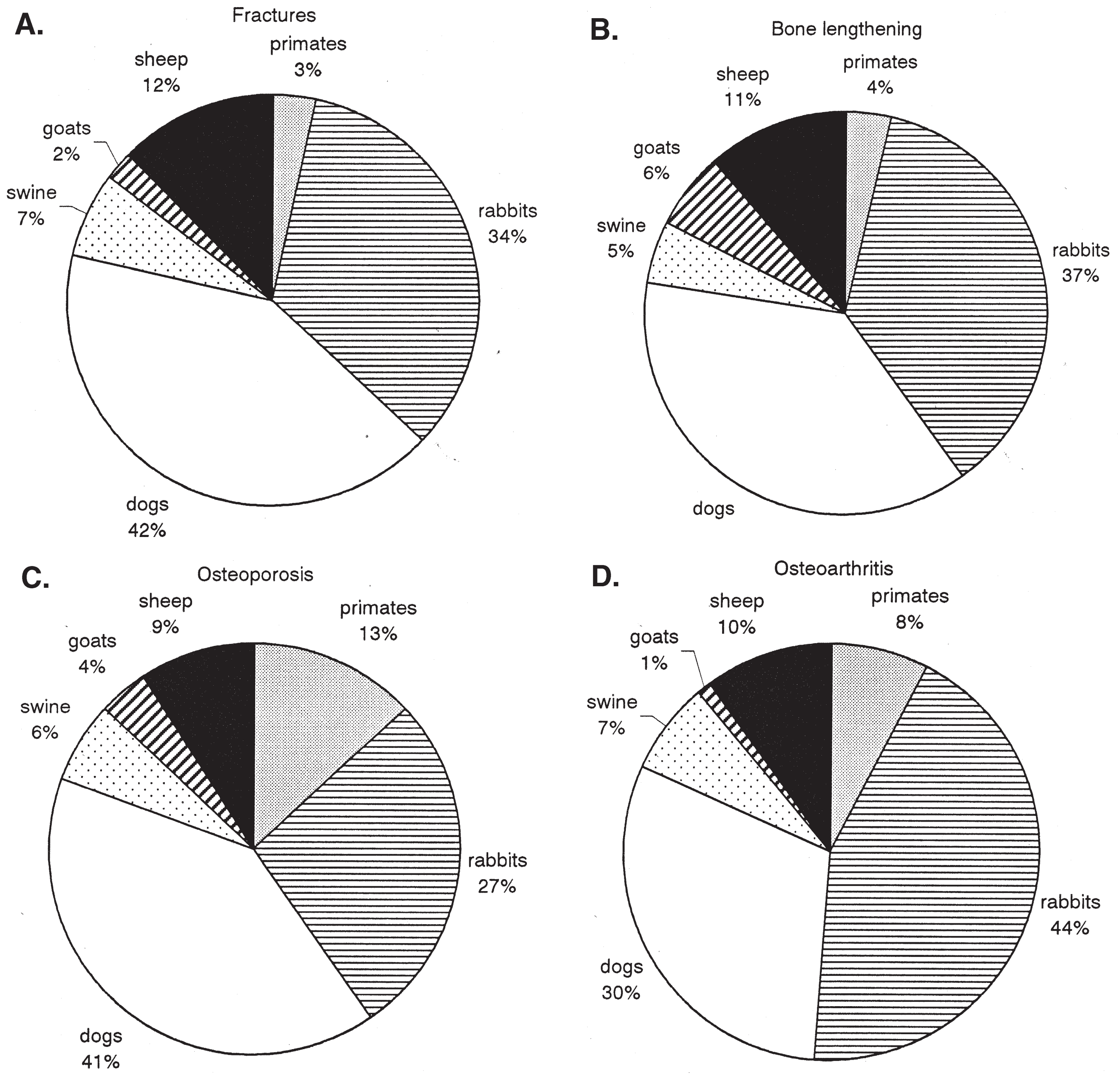

Figure 3. Diagrams showing use of non-human primates, rabbits, dogs, swine, goats, and sheep for four orthopedic pathologic conditions during the decade 1990–2001: (A) fractures; (B) bone lengthening; (C) osteoporosis, and (D) osteoarthritis.

the four main orthopedic pathologic conditions, according to the references found for the decade 1990–2001. Current research has highlighted limited use of sheep, which is quoted only in nine to 12% of the publications reviewed, versus that of dogs and rabbits, quoted in about 37 and 35% of the publications, respectively.

As far as non-human primates are concerned, they provide an excellent model thanks to their analogy with humans, but they are not cost efficient, require stringent controls, and could cause severe zoonotic diseases, as well as the ethical pressures of using this species. Non-human primates have growing and adult skeletal phases, and bone mass peaks at about 10 years in cynomolgous macaques, rhesus monkeys, and baboons. They have been used when regarded as being definitely necessary, above all for studies in the field of osteoporosis, bone ingrowth, bone repair, cartilage repair, or osteoarthritis (7).

For a long time, the dog has been considered the in vivo model closest to the human condition, except for non-human primates. It has had a dominant role in orthopedic research and has growing and adult skeletal phases (7). However, it should be remembered that such an extensive knowledge of this animal model derives from the fact that for almost 50 years, orthopedic researchers have preferred to use scientific findings from the veterinary field and instrumentation and medical devices used for treatment of this animal species. Therefore, the dog has become the only large-sized animal species to be studied extensively in research laboratories.

Nevertheless, the use of primates and dogs is decreasing in the European Community due to the emotional impact related to these kinds of animals and to the legal aspects involved in their experimental use. Moreover, there is now general agreement that there is no specific reason for dogs to be used when goats and sheep are also available for most orthopedic animal studies (7).

The pig presents some physiologic analogies with humans and has growing and adult skeletal phases, but some problems limiting its use can be associated with long-term orthopedic studies, such as its rapid body growth and weight, and its difficult handling. Smaller breeds are available, but they are more expensive and sometimes difficult to recruit.

Sheep are becoming popular as animal models in orthopedic research, especially as an alternative to dogs. However, a comparison with dogs still reveals lack of basic data. Because of the previously mentioned considerations, the purpose of the study reported here was to outline some fundamental aspects to be carefully evaluated when proposing the sheep as an experimental model in orthopedic research, to avoid errors in assessment of results due to incomplete knowledge of the animal. The characteristics highlighted herein are important when selecting sheep rather than the most commonly used species (rabbits and dogs), and can be classed into factors strictly related to bone anatomy and formation, and factors strictly affecting bone physiology, such as gastrointestinal mineral and vitamin absorption, and the reproductive cycle.

Sheep. (i) General aspects. Domestic sheep are placid animals and, because of their size, can easily be handled. They can easily be studied as an experimental animal (8). A literature search of scientific publications (MEDLINE) indicated that, during the decade 1990–2001, sheep were used to study numerous musculoskeletal pathologic conditions, such as repair of fractures and articular ligaments, limb lengthening, and treatment of osteoarthrosis and osteoporosis. Other musculoskeletal diseases studied in sheep include muscular disorders, osteomyelitis, spinal diseases, and biomaterial evaluation following International Standard Organization 10993-6 (9) (Fig. 4).

Sheep are quite similar in body weight to humans, and sufficiently large to allow serial sampling and multiple experimental procedures. They reach sexual maturity between seven and 12 months of age (average age: nine months), with slight variations between breed. At sexual maturity, the animal is not mature from the skeletal point of view yet, and growth ends a long time after puberty (Table 1).

Since there is no animal model with the same characteristics as those found in human beings, and sheep are becoming popular as in vivo experimental models in orthopedic research, we should now investigate some important aspects that could influence the results obtained when using this animal for this kind of research.

(ii) Bone anatomy. The musculoskeletal system of the sheep does not provide a direct comparison of experimental results from orthopedic research in clinical practice, especially if the biomechanical aspect is predominant. This applies to studies involving the spine and shoulder girdle, and the same is true for all of the animal species, except for non-human primates. However, the sheep is often used as a model for in vitro and in vivo experiments on intervertebral disks, fusion techniques, and spinal implants (10-12).

Accurate anatomic and biomechanical studies of the ovine spine have been published and establish which regions can be used as a model for the human spine. Briefly, the researchers conclude that, from an anatomic point of view, the gross structure of the thoracic and lumbar parts of the spine are the most appropriate, whereas the distal portion of the cervical part of the spine and thoracolumbar junction have biomechanical similarities with the human spine (10).

In their extremely accurate work with sheep, Wilke and coworkers (1997) studied the anatomic dimensions of the vertebral body, pedicle, spinal canal, spinous and transverse processes, and articular facet and intervertebral disk for comparison with human data. Their database provides information regarding the anatomy of three- to four-year-old sheep. Regional trends are similar for most measurements, but the thoracic and lumbar regions appear to be the suitable anatomic sites for spinal instrumentation (13).

The shoulder girdle of the sheep has been selected for ex vivo and in vivo studies because of the similarity between its infraspinatus tendon and the human supraspinatus tendon (14, 15). In sheep and humans, the insertion of a normal tendon into bone consists of four layers: tendon, noncalcified fibrocartilage, calcified

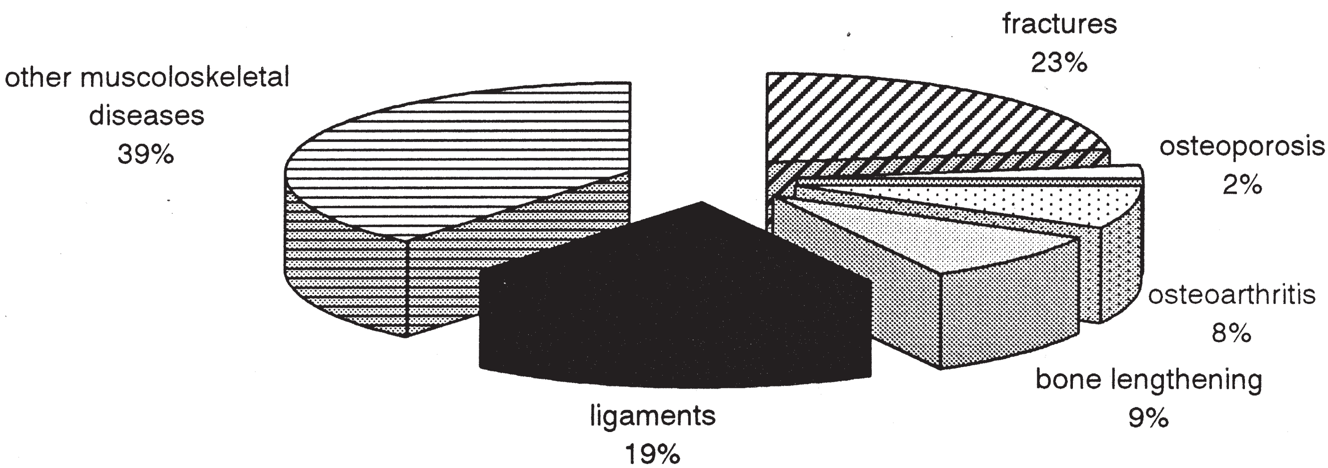

Figure 4. Average percentage use of sheep during the decade 1990–2001 in the field of orthopedics.

Table 1. Closing times of the ossification centers of the sheep

Bone	Site	Age
Maxilla		Long time before birth
Mandible		before birth (consolidation between the two bones is never complete)
Vertebrae	Body epiphysis	4-5 years
Scapula		5-7 months
Humerus	Proximal end	25-36 months
	Distal end	3-4 months
Radius	Proximal end	3-6 months
	Distal end	23-30 months
Ulna	Proximal end	25-35 months
	Distal end	26-32 months
Metacarpus	Distal end	16-18 months
Phalanx I	Proximal end	7-10 months
Phalanx II	Proximal end	6-8 months
Coxa	Iliac crest	$4^{1}/_{2}$- 5 years
Femur	Proximal end	20-26 months
	Distal end	18-26 months
Tibia	Proximal end	20-26 months
	Distal end	12-18 months
Calcaneum	Apex	36 months

fibrocartilage, and bone (15). The inconvenience of immediate full weight bearing due to quadrupedal walking of the sheep, could be avoided by adopting specific strategies (i.e., hanging devices or a hard rubber ball fixed under the hoof) (15). To the best of our knowledge, no other animal species has ever been used for the study of surgical techniques in massive rotator cuff tears.

A detailed comparative description of the surgical anatomy and approaches to the stifle joint in sheep was published by Allen and co-workers (16). Some unique features were described: presence of the extensor digitorum longus muscle on the craniolateral aspect of the stifle joint, absence of a cranial menisco-femoral ligament in the caudal joint space, and attachment of the patellar tendon to the cranial pole of the patella. However, the anatomy of the stifle joint proved to be similar to that of the human knee joint. Therefore, this joint may be considered by researchers who increasingly use sheep for studies on replacement of cruciate ligaments, collateral ligaments and menisci, treatment of chondral and osteochondral defects, and osteoarthrosis. Also, it may be of value in the use of hemiarthroplasty and arthroplasty.

(iii) Bone structure and pathophysiology. Aerssens and coworkers (17) studied the interspecies differences in bone composition, density, and quality, by comparing bone specimens from human female adults, dogs, cows, sheep, pigs, and chicken. Although the study had several limitations (small number of animals per species, animal strains not codified), the authors concluded by saying that the selected bone parameters reveal many interspecies differences for trabecular and cortical bone, and therefore, there is no ideal animal bone to be used for all orthopedic studies. However, the parameters investigated confirmed that dog bone best approximates human bone. Some similarities between humans, dogs, and sheep were found in trabecular bone, whereas the main differences were observed in cortical bone with ash, hydroxyproline, extractable protein, and insulin-like growth factor (IGF-I) content (17).

Bone mineral composition also was studied by Ravaglioli and co-workers (18), who compared values for humans, cattle, sheep, and dogs. Data were standardized by coupling the age of animals and humans with their fractional age (current age of subject/average age value calculated for the natural life span). Referring to age, the authors asserted that bone mineral composition does not appreciably differ between humans, cattle, sheep, and dogs, except for the early stages of physiologic growth (18).

In sheep, cortical bone contains fewer Haversian canals than does human cortical bone, and sheep seem to have a rate of bone healing that approximates the human rate (5). However, bone-regenerating capacity in any animal species is recognized to be faster than in that in humans. Subsequently, strategies for selecting an experimental animal model also require clear understanding of spontaneous bone defect healing, to correlate experimental data obtained from human beings. Interspecies differences may be overcome thanks to the knowledge of critical size defects (CSD), defined as "the smallest size intraosseous wounds in a particular bone and species of animal that will not heal spontaneously during the life time of the animal" or as "defects that have < 10% bony regeneration during the lifetime of the animal" (19). The CSD in each species are related to many factors, such as breed, age, skeletal site, presence or absence of fixation systems, or kind of fixation, but the most important factor seems to be the relation between bone defect and size of the selected bone segment (19, 20). Therefore, researchers usually create CSD or larger defects in cortical and trabecular bone of animals; in particular, defects that are 1.5 to 2.0 times the length of the shaft diameter are created in cortical diaphyseal bone, which often result in nonunions (5, 6). In sheep, 25- to 40-mm-long segmental defects created in the femur or tibia do not heal spontaneously (19, 21-24). Diaphyseal defects 21 mm long in the femur (25), 3 mm long in the radius of 10- to 15-kg animals (20), and 30 mm in the ulna of larger animals (26), are considered as CSD in dogs. Segmental defects of 10 to 20 mm are often created in the ulna or radius of rabbits (27-29). Finally, 6-mm femoral defects and 5- to 11-mm radial defects do not heal spontaneously in rats (30, 31). Therefore, cancellous bone defects having a diameter of 10 mm in sheep, 6 mm in rabbits, 2 mm in rats, and 10 mm in dogs are usually created (32-35).

Eitel and co-workers (36) studied bone remodeling in animals (dogs, sheep, rabbits, rats) and in humans by use of histologic evaluation of primary (evidenced by the presence of primary osteons arranged both in parallel and perpendicular to the long axis of the bone) and secondary bone (evidenced by the presence of secondary osteons almost totally arranged in parallel to the axis of the bone and surrounded by distinct cement lines). The authors asserted that rats, rabbits, and sheep all have a large primary bone structure, whereas dogs and humans have a large component of secondary bone at maturity. Subsequently, the adult dog, unlike the sheep, was found to have a substantial complement of secondary bone and, therefore, was considered a superior experimental model relative to human bony regeneration. On the contrary, some authors stated that bone remodeling activity in sheep resembles that in humans (37-39).

Bone modeling is the acquisition and transformation of overall bone form and shapes by differential variation of local growth rates, and occurs in rodents. Bone remodeling is the normal and more or less continuous reshaping of bone by resorption and redeposition (40), and occurs in dogs, sheep, primates, and adult humans. For preclinical and clinical evaluation of agents used in prevention or treatment of postmenopausal osteoporosis, it has been suggested to use the modeling and remodeling animal models (41).

The sheep has been widely investigated and used in the study of altered bone metabolism during hormone deficiency, to evaluate the effects of various therapeutic drugs on bone tissue. The ovariectomized sheep has an increase in bone formation beginning at 10 weeks and persisting until six months after surgery

(37, 38, 42-46). Bone calcium deposition reaches a peak at about one year of age, and thereafter it decreases markedly, leveling off at about nine years of age (47).

In humans, peak bone mass is attained at approximately 30 years of age, and is followed by an age-related loss that accelerates toward menopause and advanced age.

According to Aufdemorte and co-workers (48), the sheep represents an alternative to other animals in the study of osteoporosis. However, it has differences in gastrointestinal and endocrine physiology that should be investigated and taken into account in the experimental design, to determine whether they may depend on the seasonal components or the severe depression of animal bone turnover due to day length and hormonal changes (48). Moreover, it should be noted that seasonal changes in bone mass also have been reported in elderly women (49), who experience the greatest decrease in clinically measurable bone mass in winter. Also, Rodgers and co-workers (50) evaluated the various kinds of animals that can be used as in vivo models when studying osteoporosis. The sheep was considered as a non-primate model of osteoporosis by Hartke and co-workers (51), who described the various aspects to be investigated when using this animal as an in vivo model.

A wider consideration was given to the general aspects of the physiology of the sheep by Bellino and co-workers (52), who described not only the biological characteristics of its bone constitution, but also its physiologic aspects (52). A resemblance between the iliac crest of sheep and humans was reported by Pastoureau and co-workers (38) and Turner and co-workers (39).

(iv) Mineral metabolism. Normal plasma calcium (Ca) concentration is 2.5 mmol/L, and normal range is usually reported to be 9 to 12 mg/100 ml for sheep and humans. Plasma Ca concentration depends on the amount and efficiency of intestinal absorption, skeleton development and mobilization, concentrations of parathyroid hormone and calcitonin, and amounts of excretion in intestinal tract, urine, fetus, and milk. In sheep when there is an absence of vitamin D in the diet, < 20% of the ingested Ca is absorbed, whereas a 50 to 80% intake may be absorbed in the presence of adequate vitamin D (53). Dietary Ca, phosphorus (P), and vitamin D are absorbed according to metabolic requirements, vary with age and body weight, and are affected by pregnancy, lactation, fetal number, and level of milk production during lactation (Table 2) (54, 55). The absorption of Ca in sheep mainly occurs in the duodenum, where it is favored by the low pH, and to a certain extent in the jejunum, the terminal portion of the ileum, and the large intestine (56-58).

However, various factors reduce Ca absorption from the gut, such as inadequate intakes and factors affecting salivary secretion, which is the main source of Na and P for the rumen. Absorption of Ca from the rumen is unaffected by intraruminal K concentration, but it increases if intraruminal P concentration increases. Finally, Ca is excreted in the form of urinary and fecal Ca; the latter may be endogenous (Ca secretion into digestive tract) or exogenous (undigested Ca from the food). Fecal endogenous loss of Ca is not constant and increases with the level of feeding. In sheep, urinary excretion of Ca is generally low, remains fairly constant, and is unaffected by changes in absorption, unlike that in non-ruminants, which absorb more than they require and excrete the excess in the urine.

According to Ford and co-workers (54) and Hornby and co-workers.(55), the main differences in terms of Ca metabolism between

Table 2. Daily calcium, phosphorus, and vitamin D requirement in sheep

Category	Calcium*	Phosphorus*	Vitamin D†
Sheep housing	0.30	0.28	250-300
Gestation (first period)	0.27	0.25	
Gestation (last period)	0.24	0.23	
Lactation	0.52	0.37	200
Rams (> 40 kg)	0.30	0.28	200
Lambs (10-30 kg)	0.40	0.27	
(30-55 kg)	0.30	0.20	150

(modified by Blood Henderson.)
*Percentage of the total alimentary ration.
†IU/kg of dried substance.

the sheep and human lie in regulation of absorption. In sheep, such regulation is precise and Ca is absorbed only according to the requirements of the body, which means that absorption is variable but excretions are reasonably constant (54, 55). In humans, this situation is reversed: absorption is controlled only by the availability of Ca in the diet and the Ca-to-P ratio. This does not involve any major differences in the homeostasis of Ca between the two species and direct comparison can still be made, unless the tested drug is administered orally and absorbed along with Ca.

However, all factors interfering with the Ca-to-P alimentary ratio may cause alterations in the skeletal system of sheep. Among the numerous factors to be assessed, the geographic area of origin is important, because it affects light cycle and food quality. In fact, animals that are housed indoors during most of the year, are rarely exposed to the sun ultraviolet rays and their forage has low content of vitamin D. Therefore, dietary C and P absolute content should be higher for these animals than for those from other areas. Sheep are often artificially fed cereals and a grass-hay diet containing little Ca and much P; subsequently, secondary Ca deficiency may develop, worsened by vitamin D deficiency due to indoor housing.

In our opinion, studies on osteoporosis, using sheep models, should pay particular attention to the quality and composition of the pasture or herbal composition of the commercial pellet. In fact, phytoestrogens and their metabolites should be carefully controlled in ruminants, since they have deleterious effects not only on reproduction conditions, but also on bone morphology and growth. The leguminous family with estrogen activity has positive effects on animal growth thanks to formononetin, the main isoflavone in subterranean clover (*Trifolium subterraneum*) and red clover (*Trifolium pratense*), and in lucerne (*Medicago sativa*) (59).

The effects of estrogenic activity by phytoestrogens on the reproductive systems in plants, are well described for this animal species (60-62), whereas scientific data are not available on the effects of phytoestrogens on bone tissue composition in sheep ovariectomized for the study of osteoporosis.

(v) Influence of the reproductive system on bone. Like all animals, sheep do not have a clear-cut menopause at mid-life, characterized by accelerated bone loss similar to that in women. However, Dorset and Merino ewes have an estrous cycle longer than that of any other breed. The reproductive cycle during the breeding season (10 months) is strikingly similar to that of women, with an estrous cycle of 14 to 20 days, averaging 16.5 days. Therefore, sheep may prove to be more sensitive to estrogen deficiency than any other animal with less frequent estrous cycles, such as the dog.

Conclusions

There is no animal model presenting with the same anatomic, biochemical, physiologic, and biological characteristics as those found in human beings. Useful data for treating orthopedic patients are based not only on good planning and study design, but also on perfect knowledge of the animal used and of the differences between the model and the human being.

Orthopedic studies require animal models with limbs and skeletal segments of adequate size, as similar as possible to those of human beings, and sheep are quite similar in body weight to humans. However, we should consider the peculiarity of their musculoskeletal system, which does not provide direct comparison of experimental results from orthopedic research in clinical practice, especially if the biomechanical aspect is predominant. In our opinion, studies on bone metabolism, using sheep models, should pay particular attention to the quality and composition of the animal diet. In fact, phytoestrogens and their metabolites should be carefully controlled in ruminants, since they have deleterious effects not only on reproduction conditions, but also on bone morphology and growth.

Appendix:

MEDLINE Search Strategy

1. Explode medical subject heading (MESH = mh) “mammals” and inside select the following subheadings (artiodactyla [ruminants; swine], carnivora, lagomorpha, perissodactyla [equidae], primates, rodentia [guinea pigs; muridae]) obtaining the following strategy: cattle(mh) OR sheep(mh) OR goats(mh) OR swine(mh) OR dogs(mh) OR cats(mh) OR rabbits(mh) OR horses(mh) OR primates(mh) OR guinea pigs(mh) OR mice(mh) OR rats(mh);
2. Fractures(mh) OR osteoporosis(mh) OR osteoarthritis(mh) OR “external fixators”(mh) OR “internal fixators”(mh) “ligaments, articular”(mh) OR “bone lengthening”(mh) OR “muscoloskeletal diseases”(mh). Limits: only items with abstracts;
3. “Disease models, animal”(mh) OR “animal diseases”(mh);
4. 2 NOT 3. Limits: enter date from 1970–2001;
5. 4 AND 1;
6. 4 AND one of the mh reported in 1;
7. 2 NOT 3. Limits: enter date from 1970–1979;
8. 2 NOT 3. Limits: enter date from 1980–1989;
9. 2 NOT 3. Limits: enter date from 1990–2001;
10. One of mh reported in 1 among: sheep(mh), goats(mh), swine(mh), dogs(mh), rabbits(mh), primates(mh); one search for each mh;
11. 10 AND 7;
12. 10 AND 8;
13. 10 AND 9;
14. One of the mh reported in 2 among: fractures(mh), osteoporosis(mh), osteoarthritis(mh), “external fixators”(mh), “internal fixators” (mh),”ligaments, articular”(mh), “bone lengthening”(mh), “musculoskeletal diseases”(mh); one search for each mh; Limits: enter date from 1990–2001, only items with abstracts;
15. 14 AND 10.

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